maintenance of the endoplasmic reticulum as tubules and sheets. Is it possible that a similar protein(s) may be responsible for the tubular structure of mitochondria?

Overall, Fu *et al.* [2] have identified a protein that binds mitochondria to microtubules and serves as a foundation for a novel mechanism for the control of mitochondrial position and movement that is independent of motor proteins but dependent on microtubule dynamics. This study underscores the importance of mitochondrial positional control in mitochondrial inheritance, and raises questions regarding the mechanisms that control the tubular structure of mitochondria. Although mmb1p does not appear to be conserved in mammalian cells, mitochondria are maintained as uniformly distributed tubular structures in many cells that have dynamic microtubules. Therefore, it is possible that the position and movement of mitochondria or other organelles in other cell types may be controlled by mechanisms that are independent of motors but dependent on microtubule dynamics.

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Sex Determination: Switch and Suppress

The transcription factor Dmrt1 regulates male sexual development from flies and worms to humans. A newly discovered function is to suppress female differentiation in the testes. Thus, the gonadal fate decision is not final but has to be actively maintained throughout life.

Amaury Herpin and Manfred Schartl*

Sex determination — the decision whether the bipotential gonad anlage will become a testis or an ovary — is a tightly controlled and highly complex developmental process. Twenty years ago, the *Sry* gene was discovered — the male sex determining gene encoded on the Y-chromosome of mammals [1]. SRY acts on the top of a genetic cascade of transcription factors and signalling molecules that operates from the somatic cells of the undifferentiated gonad to initiate the differentiation of these cells towards a functional testis [2]. A lot of evidence suggested that the female fate is the default fate, which needs to be suppressed to allow for *Sry*-triggered male differentiation. Furthermore, the early decision towards male or female development was viewed as final. However, *Sry* is not widely conserved and is not even present in non-mammalian vertebrates. Indeed, most other genes at the top of the sex-determination cascade are not conserved in evolution, while genes further downstream have kept their function and position in the network over longer evolutionary periods [3,4]. Now, however, new results challenge our basic notions of the function and evolution of the sex-determination pathway.

These results come, surprisingly, from a gene that was regarded as one of the 'underdogs' of sex determination because of its subordinate role in the cascade; *Dmrt1*, a transcription factor of the DM domain family, is the most highly conserved member of the sex determination network, having homologues even in worms and flies [5–7]. And it is the most downstream 'worker' in the genetic hierarchy. *Dmrt1* knockout XY mice are born as males, although their testes later develop abnormally [6], leading some to rate it as a less important sex differentiation gene. Now, work from the Zarkower and Bardwell labs [8] changes not only our view on DMRT1 but also corrects the general picture of sexual development. Using intricate genetic mouse models Matson *et al.* [8] show that male sex determination is not a permanent choice and that *Dmrt1* is crucial for maintenance of testicular function. Integrating these findings with recent work on other sex determination genes [9–11] leads to an exciting new picture of how the male or female identity of the gonad is established and maintained.

Sex Suppression

To understand the role of DMRT1, Matson and colleagues [8] carefully re-investigated the abnormally developing postnatal testes of *Dmrt1* knock-out male mice [6]. Already four weeks after birth, they observed in mutant seminiferous tubules cells expressing FOXL2, a female-specific transcription factor for granulosa and theca cells (Box 1). Also, in conditional knockout mice, where *Dmrt1* was specifically ablated either in foetal Sertoli cells (Box 1) or even in the testes of adult males, FOXL2 expression was induced. This strongly indicated that the role of DMRT1 is to prevent FOXL2 expression. Concomitantly, a rise in the expression of feminizing genes (*Rspo1*, *Wnt4* and *Follistatin*) was observed together with the loss of expression of SOX9, a major component of the male determining cascade (as well as of SOX8, which is likely to act redundantly with SOX9). As a consequence of the loss of *Dmrt1* function in Sertoli cells and the upregulation of the female genetic programme by activation of the FOXL2 and WNT4/β-catenin pathways, the male-specific Sertoli cells transdifferentiated into female-specific granulosa cells. In this environment also theca cells appeared, estrogen was produced and the germ cells (Box 1) became feminized. These findings suggest that an important function of DMRT1 in male development is to actively and continuously suppress female gonad fates, a role that is at odds with the classic view of sex determination as an irreversible switch.

This newly found role of DMRT1 in maintaining male fate by suppressing female development has a direct counterpart in female gonads. Deletion of *Foxl2* in ovarian follicles of adult XX mice immediately leads to up-regulation of testis-specific genes, including the direct SRY target *Sox9* [11]. In consequence, ovarian granulosa and theca cells transdifferentiate to testicular Sertoli and Leydig cells (Box 1), respectively [11]. Obviously, similar to DMRT1, FOXL2 has to inhibit the testis differentiation programme throughout life. It does so mainly through active repression of *Sox9* regulatory sequences that are required for its expression in the testis [11,12]. Interestingly, one of the most strongly upregulated genes in such sex reverting gonads is *Dmrt1*.

Box 1

Important cell types in the mammalian gonad.

Primordial germ cells give rise to the gametes, eggs or sperm that give rise to the next generation. They migrate into the gonad anlage from their often distant sites of origin. Primordial germ cells continuously express genes that are associated with the maintenance of an undifferentiated pluripotent state. Depending on the gonadal environment, they differentiate into either meiotic oocytes or as prospermatogonia.

Somatic cell types in the ovary

Granulosa cells are the somatic steroidogenic cells of the sex cord and are closely associated with the developing oocyte. Their major function is the production of sex steroids.

Theca cells are stromal cells forming a layer outside the developing ovarian follicle in which the oocytes mature. Theca cells and granulosa cells influence each other in terms of morphology, structure, growth, and function. Theca cells produce mostly androgens, which the granulosa cells convert into estrogens.

Somatic cell types in the testis

Sertoli cells are somatic cells that associate with germ cells and nurture their development into sperm. They are the first cell type to differentiate within the gonad from bipotential precursors of the bipotential supporting cell lineage. Pre-Sertoli cells are defined as nonpolarized, dispersed somatic cells that express Sry and/or Sox9, whereas a Sertoli cell is polarized, resides within the testis cord and expresses Sox9. Pre-Sertoli cells produce prostaglandin D2, which, via Sox9, recruits other cells to the Sertoli cell fate.

Leydig cells form in the interstitium of the testis. These cells release steroid hormones (androgens) to establish and maintain secondary male sex characteristics. Leydig cells originate, at least in part, in the embryonic kidney.

Peritubular myoid cells form a single layer of flattened cells surrounding the Sertoli cells. They build the testis cords in conjunction with Sertoli cells and promote the movement of mature sperm through the seminiferous tubules of the adult testis for export to the seminal vesicles.

The emerging picture that the primary sex-determining decision is not final, but has to be affirmed life-long by suppressing the opposing sex differentiation programmes, is supported by two earlier findings: first, the loss of *Sox9* and *Sox8* in Sertoli cells after sex determination [9] causes a phenotype similar to the one reported for *Dmrt1* by Matson *et al.* [8], including the up-regulation of early ovary-specific markers. Second, when ß-catenin expression was up-regulated in Sertoli cells by deleting *Wt1*, another component of the male sex-determining cascade, a female programme could be evoked [10]. Again expression of the female marker *WNT4* and altered Sertoli cell identity were observed.

Altogether it is tempting to speculate that similar mechanisms are central for both induction and maintenance of the sex-specific gonad phenotypes. In such a two-step model (Figure 1), the first decision that directs gonadal development towards male or female fate is determined by the ability of SOX9 to establish an auto-regulatory feed-forward loop [13]. At this stage, at least in mammals, the role of DMRT1 seems to be minimal as *Dmrt1* mutant males get feminized only some time after birth [8]. For the second step, the maintenance of testis cell identity, which appears to be much less dependent of *Sox9*, *Dmrt1*, and, to a lesser extent, *Sox8*, are the decisive determinants [8–10]. Thus, while primary fate determination seems to be the result of the unilateral activation of the male pathway in which SRY activates *Sox9*, the subsequent maintenance of gonadal fate can be viewed as a battle for primacy between the male regulatory gene network (*Dmrt1*, *Sox8* and *Sox9*) and the two female networks involving *Foxl2* and *Wnt/β-catenin* signalling. A critical balance between these conflicting pathways explains the bipotential properties of gonadal cells. Whether the balance is tipped into one or the other direction is a consequence of the activation of the male or female sex determination pathway in the embryo.

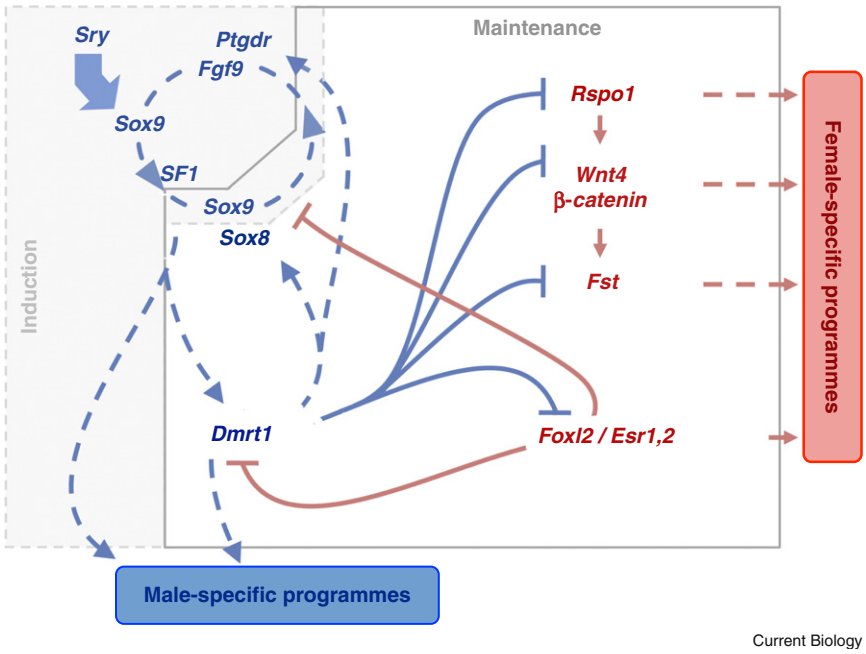

Figure 1. The *Dmrt1* gene regulatory network during gonadal induction and maintenance in mice.

Primary gonadal fate determination seems to be the result of the unilateral activation (or not) of the male pathway (blue) in which SRY activates Sox9. In a second step, maintenance of gonadal fate can be viewed as a battle for primacy between the male regulatory gene network (Dmrt1, Sox8 and Sox9) and two female networks (red) involving Foxl2 and Wnt/β-catenin signalling. A critical balance between these conflicting pathways explains the underlying property of bipotentiality in cells of the gonad. Whether the balance is tipped into one or the other direction for regulation of the cellular gonadal fate is a consequence of the activation of the male or female sex determination pathway in the embryo, which puts a lifelong imprint on this cell lineage. Model adapted from [8,11]. Dashed lines indicate potential positive regulation while solid lines indicate possible negative regulation.

Sexual Transdifferentiation

The above-mentioned papers illuminate the intrinsic molecular mechanisms of what appears to be a true transdifferentiation of an adult cell lineage *in vivo*. As a result of the conversion of differentiated Sertoli cells into apparently functional granulosa and theca cells in the feminized gonads of *Dmrt1* knockouts, expression of enzymes (CYP19A1/aromatase) critical for ovarian development through oestrogen production by granulosa cells is elevated. Also expression of oocyte-specific proteins and apparent responsiveness to gonadotropins was observed [8]. In a similar vein, upon loss of *Foxl2* in the adult ovary, the two major female-specific somatic lineages also switched fate: granulosa cells, which support oocytes, transdifferentiated into Sertoli-like cells, and the steroidogenic theca cells upregulated *Hsd17β*, the rate-limiting enzyme in testosterone biosynthesis and a Leydig cell marker, resulting in male-like blood testosterone levels [11]. The specific transdifferentiation of Sertoli/Leydig cells to their female granulosa/theca cell counterparts, and vice versa, might indicate common initial precursors for these four cell types.

This apparent transdifferentiation certainly reflects a high degree of plasticity. It also raises the question whether this phenomenon is indeed the result of a true reprogramming process. This would require that the cells pass (transiently) through a de-differentiated state — comparable to the postulated common precursor of all somatic gonadal cell types — before they switch towards redifferentiation into their cellular counterpart of the opposite sex. Actually, the observed intratubular cells that co-express SOX9 and FOXL2 as well as the persistence a few SOX9-positive cells during transdifferentiation [8] might reflect such a dedifferentiated state. Interestingly, unlike most other cell reprogramming systems, this gonadal reprogramming is not due to enforced activation of a gene expression programme. Rather, the differentiated state of gonadal somatic cells is obviously mainly under negative control, and thus reprogramming of the gonad is mainly due to a release from suppression of the alternative fate. Hence, understanding the plasticity of adult gonads might contribute in a more general way to understanding how differentiated cells can be reprogrammed, a central issue of stem cell research and regenerative medicine.

DMRT1 — a Jack of All Trades?

The observations that one of the most robustly upregulated genes in the ovary upon *Foxl2* deletion is *Dmrt1*, and that FOXL2 and DMRT1 appear to exhibit opposing effects on the *Sox9* autoregulatory loop [8,11], lead to the view that DMRT1 is essential to make a mammalian testis. Its range of action is not limited to the maintenance of the male pathway after its initiation but also largely accounts for the active repression of the two 'anti-testis' pathways of FOXL2 and WNT4/β-catenin. Consequently, mammalian sex determination may be seen as an equilibrium of antagonistic pathways for which SRY, rather than acting as a main switch for the male pathway, would just kick off the *Sox9*/*Dmrt1* feed forward loop (Figure 1). On the other hand, in the absence of the SRY impulse, the role of FOXL2, besides the activation of female-specific mRNAs, would be to avoid activation of the loop by maintaining both *Sox9* and *Dmrt1* expression at low levels (Figure 1). Furthermore, the reversion of granulosa cells to male gonad cells after deletion of *Foxl2* indicates that there isn't a default female development on top of which a male programme has to be imposed by an *Sry* enforced 'maleness' cascade to form testes.

So, how do we define the role of *Dmrt1* in the light of these new findings? It emerges that *Dmrt1* holds a key position as the master switch between the male and female cell fate of the gonad and that it flips the switch towards the male fate. If this is so, the question may be asked why such a complicated regulatory network upstream of *Dmrt1* is required to flip the switch. And indeed, there are examples that indicate that DMRT1 could do the

job alone. In chicken, and most likely all birds, *Dmrt1* has usurped the top position as the master regulator of male development [7,14]. Also, in *Xenopus laevis* and the Medakafish (*Oryzias latipes*), an additional gene copy of *Dmrt1* became the primary sex determiner [15–18].

New sex determination mechanisms are known to evolve rapidly. The inherent switch function of the *Dmrt1* gene made it obviously very suitable for selection as a new controller at the top. Not surprisingly, in other situations of plasticity, namely sex change as it occurs, for instance, in a number of fish species, the transition from one to the other sex is characterized by up-regulation of *Dmrt1* once the gonad switches towards male and *vice versa* [5].

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Gene Regulation: Implications of Histone Dispersal Patterns for Epigenetics

Histones are widely believed to carry regulatory information across cell generations. A recent study suggests limits to this model by measuring dispersal of ancestral histones in yeast.

Nicole J. Francis

Chromatin structure is widely believed to carry heritable gene regulatory information in eukaryotes, allowing epigenetic inheritance of gene expression patterns. The wide array of histone modifications has led to the suggestion that the histones themselves may be important carriers of information [1,2]. This is an attractive hypothesis because the histones are intimately associated with the DNA that they are purported to regulate, and because parental histones are known to segregate to newly replicated DNA [3]. Furthermore, for many histone modifications, the histone-modifying enzyme itself, or a protein it associates with, recognizes the modification it creates, providing a mechanism to propagate modifications (referred to as 'spreading') [4]. This hypothesis is challenged by histone disruption caused by DNA replication and transcription, as well as replication-independent turnover of histones [5,6]. Thus, understanding the extent and time scale over which histones are dispersed is central to considerations of histones as carriers of epigenetic information.

In a recent issue of *PLoS Biology*, Radman-Livaja and colleagues [7] used a genome-wide mapping strategy to measure dispersal of parental histones in yeast over several generations. To label 'ancestral' histones, a recombination system developed by van Leeuwen [8] was used to switch epitope tags on Histone H3 expressed from its endogenous locus. Histone H3 tagged with HA was expressed constitutively to label the pool of ancestral histones; recombination is then induced so that HA-histone H3 is no longer expressed, but instead T7-H3 is expressed. Thus, old (HA) histones can be distinguished from new (T7) histones. T7- and HA-tagged nucleosomes were mapped across the genome, and the ratio of HA to T7 used to monitor ancestral histones for several generations.

The ancestral histones do not show a uniform pattern across the genome as might be expected if they are mainly stable and dispersed randomly by DNA replication (Figure 1). Instead, ancestral histones accumulate at the 5′ ends of transcribed genes (which cover much of the yeast genome). A mathematical model was developed to describe the change in the distribution of ancestral histones over generations with three parameters describing histone dispersal. The first is histone turnover (replacement of ancestral histones with